様式

論 文 内 容 の 要 旨

氏 名 DAO VIET LINH

Natural rubber is widely used for gloves, tires and shock-absorbing materials in human life. Rubber trees produce natural rubber by fixing carbon-dioxide in the air and therefore natural rubber is a carbon-neutral resource. The increasing use of natural rubber has caused an environmental problem with rubber wastes after use and from rubber-processing factories. This study was conducted to solve such a problem by using microbial rubber-decomposing ability through detailed characterization and improvement of degradation system in bacteria. A couple of rubber-degrading bacterial consortia were individually enriched from the wastes of rubber-processing factories in Vietnam. Among bacterial components found in each consortium, only one strain in each consortium was responsible for rubber degradation, which was revealed by the rubber degradation activity of each component strain determined by gel permeation chromatography analysis. Based on the 16S rRNA gene sequence, the rubber-degrading strains isolated were identified as *Nocardia* sp. NVL3 and *Rhodococcus* sp. E2. Structural analysis of the rubber-degrading microbial consortia employing denaturing-gradient-gel-electrophoresis and 16S-rRNA based metagenome analysis revealed these key strains are minor components in each consortium.

The strain NVL3 grew well and gently on deproteinised natural rubber (DPNR) in liquid minimal salt (MS) medium and on a solid MS agar plate, respectively. NVL3 degraded both DPNR and synthetic poly(*cis*-1,4-isoprene) (SR) in liquid MS medium and produced aldehyde intermediates from SR, which was visualized by an aldehyde staining assay on an MS agar plate. It also grew well in liquid MS medium using both squalene and gutta-percha as sole sources of carbon, which are low-molecular polyisoprenes. By the draft genome sequencing, a single latex-clearing protein (*lcp*) gene ortholog was identified in NVL3. The genome of NVL3 also contained an extensive number of putative enzyme genes, which are expected to be involved in a rubber degradation pathway via ß-oxidation. The *lcp* gene product (Lcp), which was purified after expression of the NVL3 *lcp* gene in *E. coli*, degraded SR *in vitro* and produced aldehyde intermediates from DPNR on an MS agar plate. The strong transcriptional induction of the *lcp* gene in NVL3 only in the presence of SR was indicated by the reverse transcription-PCR (RT-PCR) and quantitative RT-PCR (qRT-PCR) analyses. The purified product of the oxidoreductase gene (*oxiI*) neighboring the *lcp* gene, which was expressed in *E. coli*, reduced the aldehyde intermediates produced by Lcp on a DPNR-containing MS agar plate. qRT-PCR analysis revealed that *oxiI* was also induced only in the presence of SR. These results suggested *oxiI* is responsible for transformation of the aldehyde intermediates.

The strain E2 grew well on DPNR both in liquid MS medium and on a solid MS agar plate. E2 degraded DPNR to aldehyde intermediates. It also degraded SR in liquid MS medium. The presence of a single *lcp* gene ortholog and an extensive number of putative

rubber-degradation enzyme genes in E2 was suggested by draft genome sequencing. The *lcp* gene product of E2, which was produced in *E. coli* and purified, degraded SR *in vitro* and generated aldehyde intermediates from DPNR on an MS agar plate. It exhibited a higher specific activity than the purified NVL3 Lcp, which was examined by oxygen consumption assay. When the E2 *lcp* gene was introduced and expressed in *Rhodococcus jostii* RHA1 the recombinant strain grew gently on a DPNR-containing MS agar plate.

Both the purified Lcps of NVL3 and E2 were able to degrade rubber *in vitro* suggesting the innate activity of Lcp on rubber degradation. In addition to NVL3 and E2 the purified Lcp of *Streptomyces* sp. K30 was reported not to have formed a clearing zone on a rubber-containing plate, suggesting that Lcp is not solely responsible for clearing zone formation. The oxidoreductase gene of NVL3 has no similarity with the *oxiA* and *oxiB* genes, which were reported to be involved in transformation of aldehyde intermediates in the strain K30. Among eight rubber-degrading actinobacteria only two strains have orthologs of *oxiA* and *oxiB*, and the enzyme genes of the remaining six strains responsible for transformation of aldehyde intermediates are wrapped in mystery. The newly-found oxidoreductase gene of NVL3 is expected to solve this mystery. This study added new findings in rubber degradation system in non-filamentous actinobacteria and provided further interests on improvement of rubber degradation system for applications.